REMARKS

Applicants appreciate the courtesies extended to their representative, Allan A. Fanucci, by Examiner Michael Borin during a telephone interview on January 4, 2010. During that interview a draft amendment was discussed. The Examiner noted that the amendment did not place the application in condition for allowance. A copy of the amendment is attached to the interview summary. Thus, the applicant has further amended the claims and is now submitting further explanation of the way the invention is conducted to show that it meets all statutory requirements as well as that is patentably distinct from the cited art. The further comments in support of the preceding are now presented herein.

Claims 109-121, as amended, and new claims 122-124 are presented herein for the Examiner's review and consideration. Claim 109 has been amended to recite a preferred embodiment, wherein the method comprises binding a first label to at least one ribosome or a fragment thereof to form a donor fluorophore; binding a second label to at least one tRNA to form an acceptor fluorophore; detecting electromagnetic radiation signals emitted when the first and second labels are in proximity, wherein the signals are obtained from the donor and acceptor fluorophores forming a fluorescence resonance energy transfer (FRET) pair, with the signals indicating progression of the synthesis of the one or more proteins; and analyzing the detected signals to identify one or more proteins being synthesized by producing a FRET signal from the FRET pair, computing a synthesis signal from the FRET signal, and interrogating a database compiled from signal data of various FRET pairs so as to identify the one or more proteins that most likely have produced the detected signals. The synthesis signal is computed by recording beginning and end points for each FRET signal time period; and computing probabilities of labeled sequences based on the type of signals recorded and the time differences of the recorded beginning and end points. Support for these changes are found in the specification in paragraph [0029] and in the section entitled Fluorescent Resonance Energy Transfer (FRET) starting at paragraph [0086] and specifically in paragraph [248] as well as in the Abstract of the published specification. Claim 111 has been amended to be consistent with claim 109.

New claims 122-124 are directed top preferred embodiments that are shown in the specification, in particular, on pages 68-72 of the specification as filed. As no new matter has been introduced by these changes and additions, they all should be entered at this time.

Claims 109-121 have been rejected under 35 U.S.C. 112, second paragraph, for the reasons set forth on page 3 of the office action. In response, claim 109 has been amended to make it clear that the method comprises binding a first label to at least one ribosome or a fragment thereof to form a donor fluorophore; binding a second label to at least one tRNA to form an acceptor fluorophore; detecting electromagnetic radiation signals emitted when the first and second labels are in proximity, wherein the signals are obtained from the donor and acceptor fluorophores forming a fluorescence resonance energy transfer (FRET) pair, with the signals indicating progression of the synthesis of the one or more proteins. The specification explains how this works for multiple donors and acceptors such that a skilled artisan would understand how the invention operates. Furthermore, the detected signals are analyzed to identify one or more proteins being synthesized by producing a FRET signal from the FRET pair, computing a synthesis signal from the FRET signal, and interrogating a database compiled from signal data of various FRET pairs so as to identify the one or more proteins that most likely have produced the detected signals. Thus, the invention is now described in a more particular way such that this rejection is no longer applicable.

Claims 109-121 have been rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth on pages 4-6 of the office action. In response, Applicant respectfully submits that the claims have been amended to cover a preferred embodiment which is completely described and detailed in the specification such that the rejection is overcome. In particular, claim 109 recites that a first label is bound to at least one ribosome or a fragment thereof to form a donor fluorophore and that a second label is bound to at least one tRNA to form an acceptor fluorophore. Also, the method includes analyzing the detected signals to identify one or more proteins being synthesized by computing a synthesis signal from the FRET pairs by recording beginning and end points for each FRET signal time period; and computing probabilities of labeled sequences based on the type of signals recorded and the time differences of the recorded beginning and end points. Finally, a database compiled from protein sequence data is interrogated to identify the one or more proteins that most likely have produced the detected signals. These method steps are disclosed in the E4 data described in the specification at paragraphs [0305] to [0324]. In addition, a short power point presentation and specific working example based on that part of the specification is now provided.

The enclosed presentation shows the results of PSM using ribosome:tRNA FRET as described in the patent specification. Single molecule FRET (smFRET) studies have been conducted to demonstrate the interaction between a ribosome labeled with Cy3 fluorescent probe (donor) on L1 ribosomal protein (Figure 1), and tRNAs some of which are labeled with Cy5 (acceptor) on the dihydrouridine nucleotides of the D-loop of the tRNA (Figure 2). The experiment is performed with a TIRF microscope, in which only fluorophores placed within 100 nm of the glass surface are detected (attached presentation, slide 2). Ribosomes programmed with an mRNA containing a biotin at the 5'-end are immobilized on a surface containing streptavidin bound to biotinylated PEG. The optics of the microscope allow separate and simultaneous detection of donor (donor channel) and acceptor (FRET channel) fluorescence arising from donor excitation. Automated software analyzes the raw video sequence (presentation, slide 3), identifies the signals from single ribosomes (slide 4) and compiles the traces from each ribosome (slide 5).

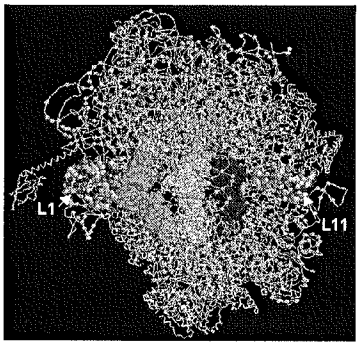

Fig. 1. Relative dispositions of proteins L11 (spheres on the right) and L1 (spheres on the left) with respect to tRNAs bound in the A (right), P (middle), and E (left) sites [Yusupov 2001]

The specific steps are shown in detail as follows:

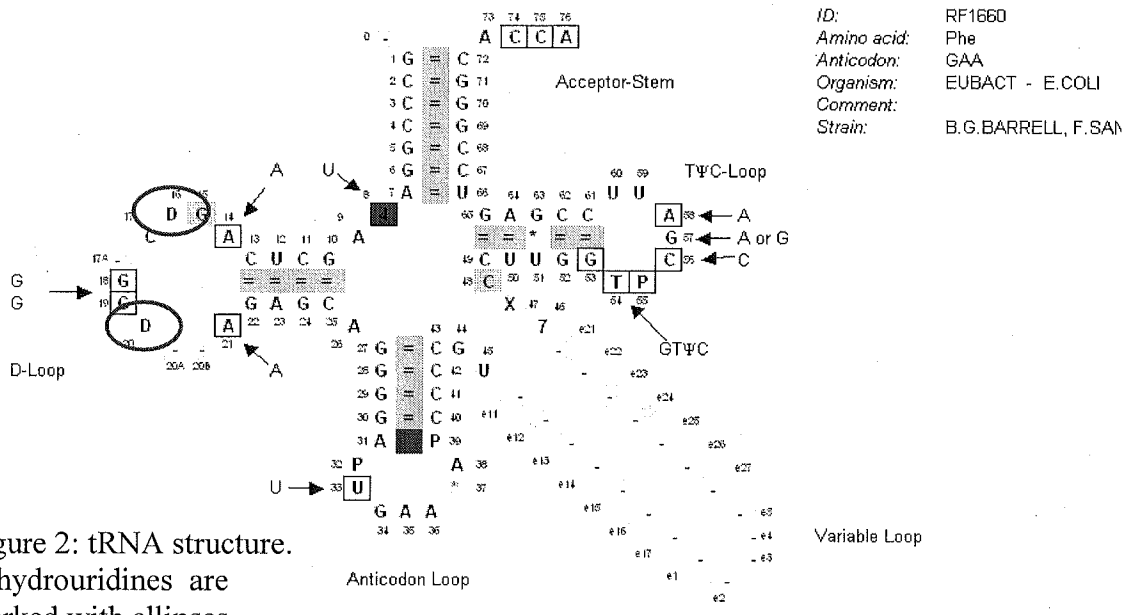

Figure 2: tRNA structure. Dihydrouridines are marked with ellipses.

Step 1: Database Compilation

Consider three short messages that are analyzed as follows:

M1: MRFVRFVRF

M2: MRRFRRFRR

M3: MRFRFRFRF

In this example, only the Arginine (R) tRNA is labeled, leading to the following PSM signal sequences:

S1: 10010010

S2: 11011011

S3: 10101010

Note: the sequence starts when the first signal (i.e., the labeled amino acid) is measured.

These signals are coded according to the number of unlabeled elements between two labels:

T1: 2,2

T2: 0,1,0,1,0

T3: 1,1,1

Note: the sequence starts at the first label and ends at the last label.

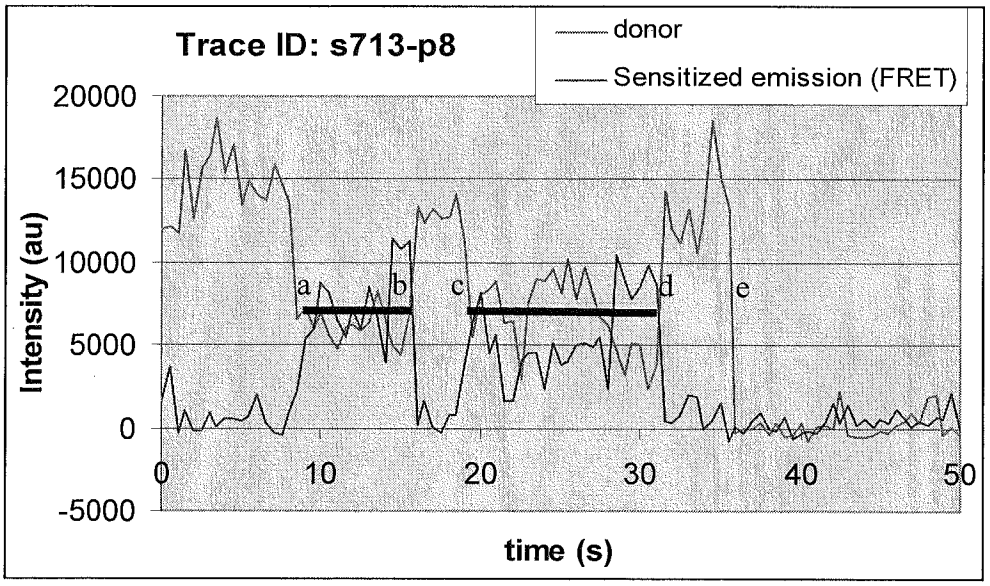

Figure 3: trace from an unidentified message (one out of M1-M3). The red (acceptor) trace is not used in the computation.

The set of coded signals (T1-T3) is entered into the database. In this simple example it is the set of full-length expected sequences. For each entry, a link is stored for the original message (T1→M1, T2→M2, T3→M3).

Step 2: message identification

Figure 3 shows a trace from a message to be identified. The trace analysis algorithm interprets the trace as follows:

Step 3: identification of binary FRET events

1. start of first FRET event, t=8
2. end of first FRET event, t=16
3. start of second FRET event, t=19
4. end of second FRET event, t=32
5. bleaching of donor fluorophore, t=36

The binary FRET signal is marked by two thick bars, a-b and c-d.

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Step 4: computation of the synthesis signal (interval timings)

Fret event timings are:

E1: (8+16)/2 = 12

E2: (19+32)/2 = 25.5

The result is a single interval, dT = 25.5-12 = 13.5

In the current experimental setup, the ribosome processing is slow due to the current parameters of the cell-free system. Also, the analysis is based on prior data of average ribosome timing for processing a single amino acid. In this simple example the "label tree" has degenerated into a single "leaf".

Step 5: computation of interval likelihoods for an interval of dT=13.5 seconds

A likelihood table is determined as follows:

1. Interval of 0 AminoAcids – 2%
2. Interval of 1 AminoAcid – 17%
3. Interval of 2 AminoAcids – 69%
4. Interval of 3 AminoAcids – 11%

Based on this data, the results show that:

- the likelihood of this interval matching T1 (2,2) is 69%

- the likelihood of this interval matching T2 (0,1,0,1,0) is 19% (2%+17%)
- the likelihood of this interval matching T3 (1,1,1) is 17%

Step 6: message identification

Conclusion: the intervals measured in this trace arise most likely from T1 based on the probabilities. And thus from message M1 which is linked to T1: M1: MRFVRFVRF. This identifies that peptide as the one that has the greatest likelihood of resulting from the measured data. And while a small scale simple model has been shown, a skilled artisan will immediately recognize that the process can be scaled up as necessary to apply to more complex molecules and larger numbers of signals. The compilation of the database and its interrogation based on the time interval matching demonstrates that the process can be conducted by a skilled artisan without undue experimentation. In view of the foregoing, the enablement rejection has been overcome and should be withdrawn.

Claims 109-121 were rejected as being unpatentable over the Odom article. The present claims have been amended to more specifically define the invention in a way that is not disclosed in or taught by Odom. As explained above, the present claims recite the binding of a first label to at least one ribosome or a fragment thereof to form a donor fluorophore; the binding of a second label to at least one tRNA to form an acceptor fluorophore; the detecting of electromagnetic radiation signals emitted when the first and second labels are in proximity due to the donor and acceptor fluorophores forming a fluorescence resonance energy transfer (FRET) pair, and the analyzing of the detected signals including the interrogating of a database compiled from signal data of various FRET pairs so as to identify the one or more proteins that most likely have produced the detected signals. As noted above, the analyzing is conducted by computing a synthesis signal from the FRET pairs based on the recording of the beginning and end points for each FRET signal time period, followed by computing probabilities of labeled sequences based on the type of signals recorded and the time differences of the recorded beginning and end points. Odom does not disclose the analysis feature of the present invention.

Odom discloses the movement of tRNA during peptide bond formation on ribosomes. Odom does not teach or suggest that synthesis signals from FRET pairs be computed based on the recording of the beginning and end points for each FRET signal time period. Instead, Odom uses fluorescent techniques and in particular the movement of fluorescent probes e.g., tRNA, to physically define the ribosomal binding sites. This has nothing to do with the time measurements of FRET signals.

The present method also provides unexpected advantages over Odom. The present invention enables the synthesis of proteins by the ribosome to be monitored in real time, in vivo as well as in in-vitro. The ribosome is engineered to carry a donor fluorophore, and tRNA and/or amino acids and/or some other part of the ribosome are either engineered to carry acceptor fluorophores. As the ribosomes mechanism processes the mRNA and tRNA molecules and synthesizes a polypeptide chain, the ribosome can be illuminated, so as to excite the donor fluorophores and thereby the acceptor fluorophores whenever donor and acceptor fluorophores are in sufficient proximity to each other. The resulting signals are detected in the manner described herein and used as a key for real-time database searching and identification of the protein being synthesized. As the claims are now directed to more specific features of these operations, the claims are now further distinguishable from Odom and the rejection based on Odom should be withdrawn.

In view of the foregoing, it is believed that the entire application is now in condition for allowance, early notice of which would be appreciated.

Respectfully submitted,

1/26/10

Date

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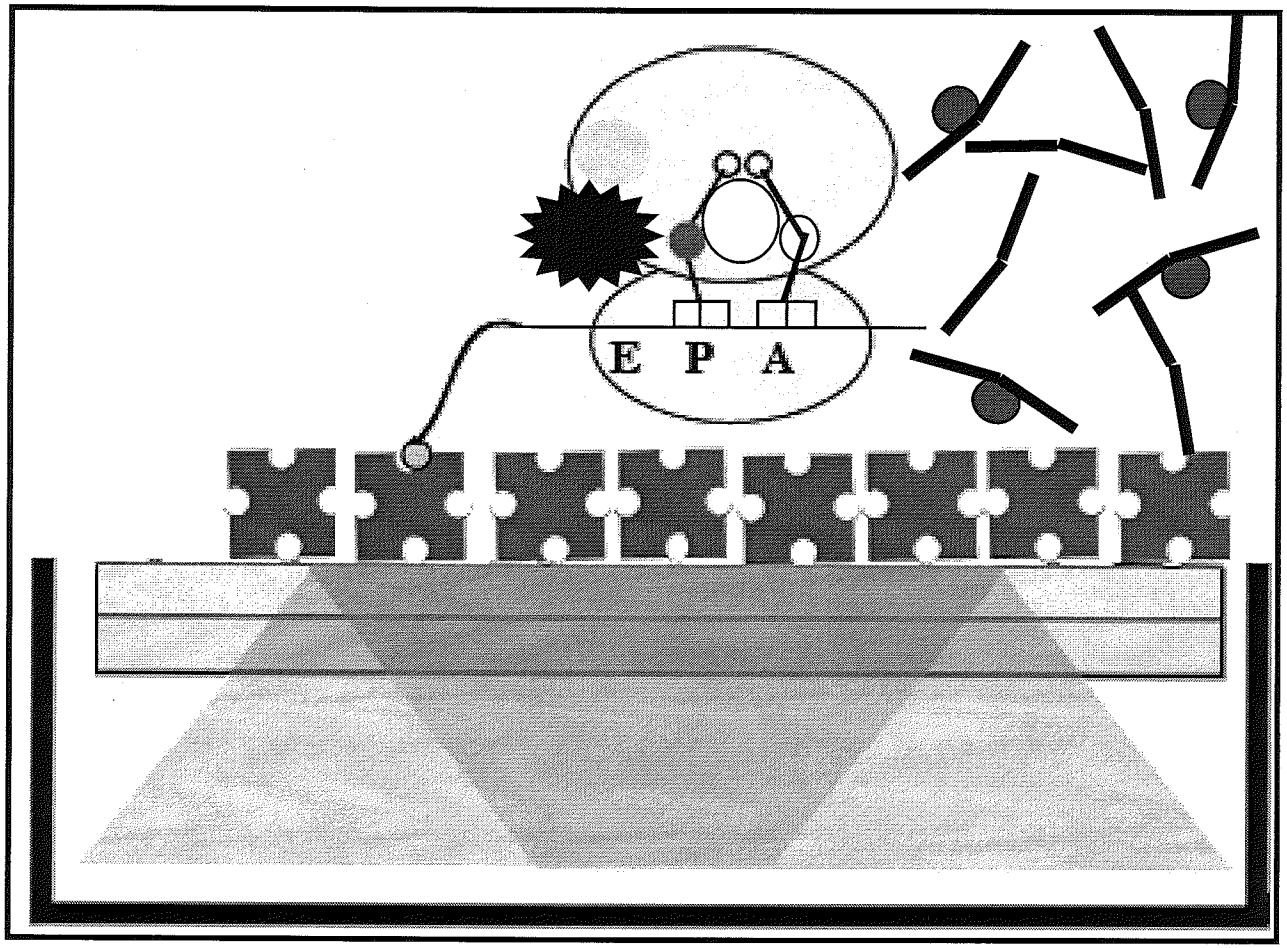
PSM-
Protein Synthesis Monitoring

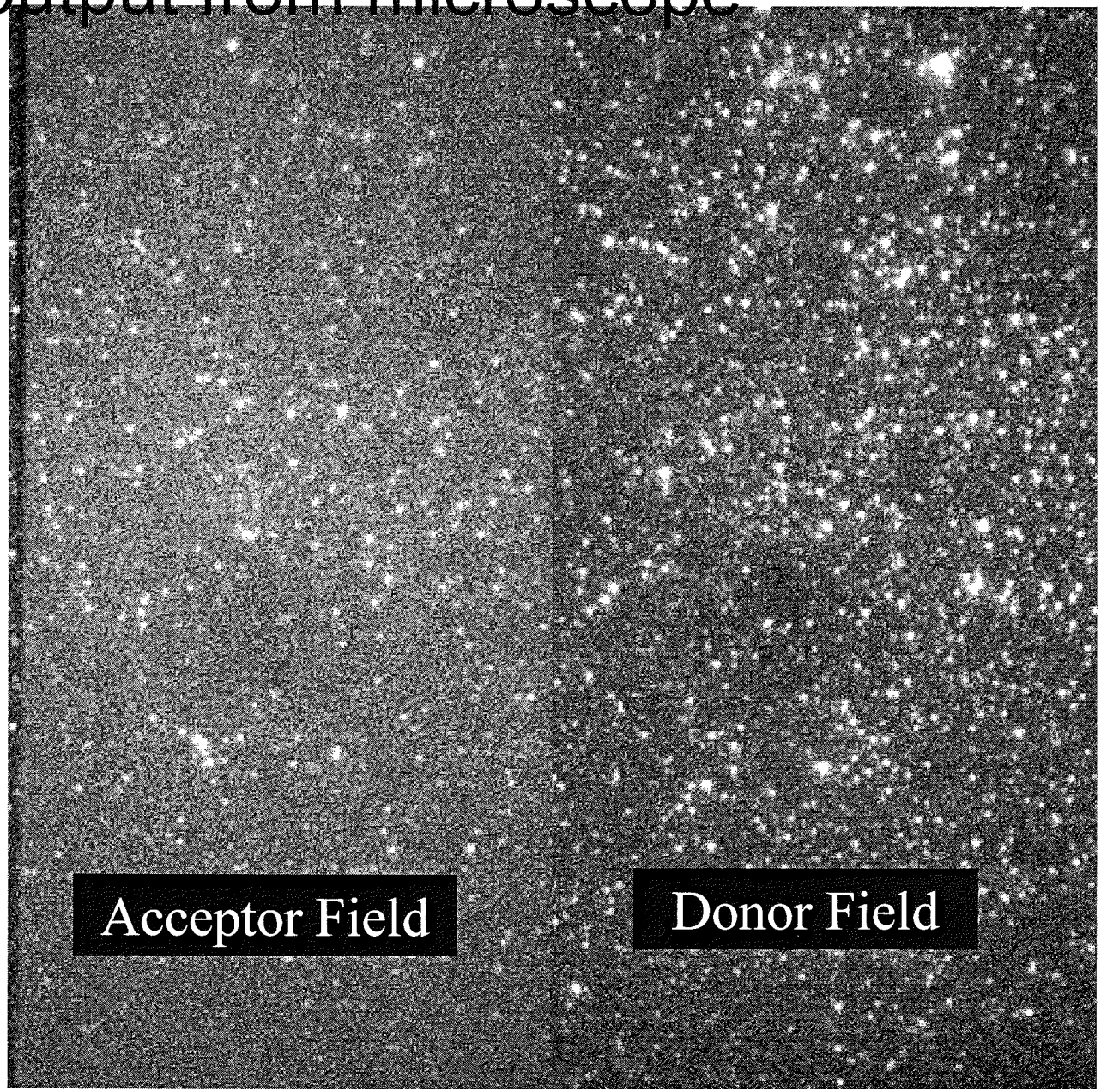
Initial results of PSM using ribosome:tRNA FRET (as described in the patent specification) with worked example

Ribosome:tRNA experimental setup

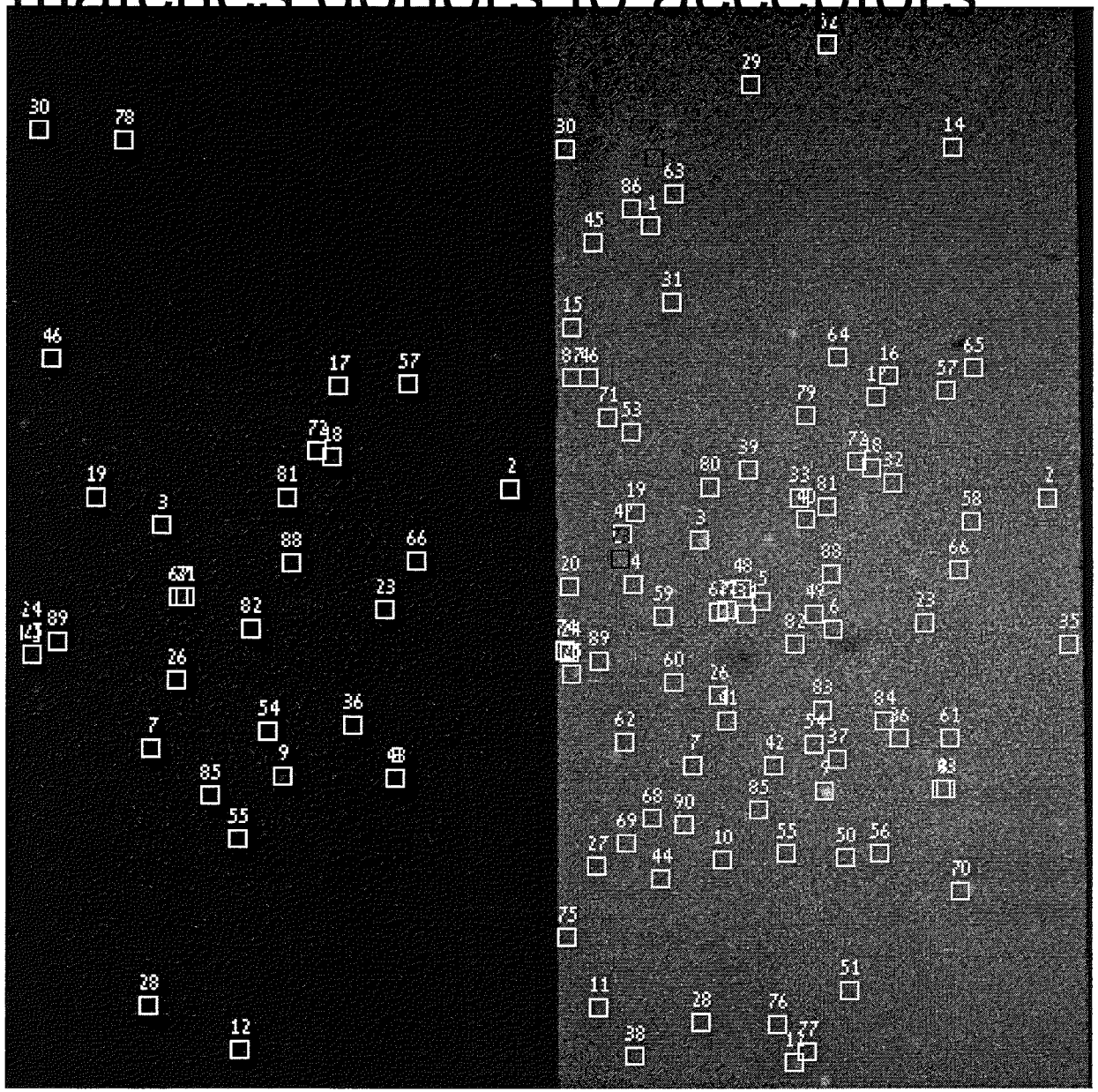


- Donor (Cy3) on ribosomal protein L1
- Acceptor on $tRNA^{Arg}$
- Message immobilized to microscope slide with TIRF illumination

Single Ribosome Movie – raw output from microscope



Software picks up spots and matches donors to acceptors



Each spot yields a trace (as below) with donor, acceptor and FRET channel intensities